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MACROMOLECULAR SYNTHESIS AND SYNTHETIC BIOLOGY

Rocklin G J, Chidyausiku T M, Goreshnik I, Ford A, Houliston S, Lemak A, Carter L, Ravichandran R, Mulligan V K, Chevalier A, Arrowsmith C H, Baker D. Global analysis of protein folding using massively parallel design, synthesis, and testing. *Science* 357; 2017:168–175.

Rocklin *et al.* undertake *de novo* protein design and stability testing on an unprecedented scale to identify sequence determinants of folding. They synthesize thousands of "miniproteins," polypeptide domains of 30–50 residues, choosing sequences on the basis of computational design for stability. Because they don't select for function, they sample a much larger swathe of sequence space than naturally occurring proteins occupy. With the use of Rosetta, the authors design sequences to fold into 4 conformations: ααα, βαββ, αββα, or ββαββ. The sequences are encoded by genes synthesized using highly parallel techniques for DNA assembly. DNA sequence libraries are expressed in yeast so that each cell displays on its surface many copies of a single protein sequence fused to an expression tag that can be labeled with a fluorophore for the purpose of fluorescence-activated cell sorting. The cells are incubated with varying concentrations of a protease, and the sequences that fold stably enough to survive proteolytic digestion at each protease concentration are ascertained by high-throughput DNA sequencing after sorting the parent cells that express them. Each protein sequence is thus assigned a "stability score" that expresses its resilience to proteolysis compared with scrambled sequences in unfolded states. With the use of this methodology, the authors measure folding and stability for >15,000 miniproteins, >10,000 point mutations, and >30,000 control sequences. They identify 2500 stably folding sequences and perform iterative rounds of protein selection and stability scoring to improve their rate of success in designing sequences for stability. This study breaks new ground in the investigation of the determinants of protein folding and *de novo* design of useful proteins.

Iwamoto N, Butler D C, Svrzikapa N, Mohapatra S, Zlatev I, Sah D W, Meena, Standley S M, Lu G, Apponi L H, Frank-Kamenetsky M, Zhang J J, Vargeese C, Verdine G L. Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nature Biotechnology* 35;2017:845–851.

The substitution of a sulfur atom instead of oxygen in the phosphodiester bond in DNA creates a phosphorothioate bond. Because phosphorothioate bonds protect against enzymatic degradation, they are used in making antisense oligonucleotide therapeutics. Regrettably, the introduction of the sulfur creates a chiral center that can exist in two stereochemical configurations: S_P and R_P. Phosphorothioate oligonucleotides are generally stereochemically heterogeneous, as they are synthesized under conditions that do not control the stereochemistry. Procedures for stereo-controlled synthesis of antisense oligonucleotides are refined in the present paper. The authors use existing nucleoside 3′-oxazaphospholidine derivatives as monomers. The reaction cycle occurs in 4 steps: 1) coupling, which proceeds *via* clean inversion of the absolute configuration of the monomer to form a trialkyl phosphite; 2) standard capping with phenoxyacetic anhydride, which acetylates the 5′-hydroxyl on failure sequences and the pyrrolidine nitrogen on the chiral auxiliary (the group on the monomer that maintains the desired chirality of the product); 3) sulfurization of the phosphorus atom on the trialkyl phosphite with *S*-cyanoethylmethylthiosulfonate to give *S*-cyanoethyl phosphorothioate with retention of the absolute configuration of the phosphorus center and spontaneous removal of the auxiliary; and 4) detritylation with trichloroacetic acid for addition of

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the next residue. This cycle is repeated for each residue. The *S*-cyanoethyl protecting groups are then removed by conjugate elimination on the resin, and finally the completed oligonucleotide is released from the resin and its nucleobase protecting groups are removed in a single step using aqueous ammonia. The stereochemistry of each phosphorothioate linkage is chosen individually by selecting the appropriate chirality of the monomer in Step 1 of each cycle. The coupling efficiency is described as "high." The authors use their methodology to synthesize stereo-pure forms of the antisense oligonucleotide Mipomersen, which knocks down apolipoprotein B-100 biosynthesis, thus reducing levels of low-density lipoprotein in the treatment of familial hypercholesterolemia. Iwamoto *et al.* demonstrate *in vivo* in mice selected sequences of chiral centers that increase the efficiency of knockdown *in vitro* and enhance the durability of phosphorothioates.

Hoppmann C, Wong A, Yang B, Li S, Hunter T, Shokat K M, Wang L. Site-specific incorporation of phosphotyrosine using an expanded genetic code. *Nature Chemical Biology* 13;2017:842–844.

Luo X, Fu G, Wang R E, Zhu X, Zambaldo C, Liu R, Liu T, Lyu X, Du J, Xuan W, Yao A, Reed S A, Kang M, Zhang Y, Guo H, Huang C, Yang P-Y, Wilson I A, Schultz P G, Wang F. Genetically encoding phosphotyrosine and its nonhydrolyzable analog in bacteria. *Nature Chemical Biology* 13;2017: 845–849.

Two papers describe methods for efficient production in *Escherichia coli* of recombinant proteins into which phosphotyrosine or a chemical analog is stoichiometrically incorporated at predetermined sites. This goal has hitherto been impeded by several circumstances. Phosphotyrosine is poorly imported into live cells because of its negative charge. It is unstable inside cells, and its presence is incompatible with translation by elongation factor Tu. Both of the present groups fulfill the goal by using expansion of the genetic code. Hoppmann *et al.* evolve a tRNA and cognate aminoacyl-tRNA synthase to incorporate tyrosine phosphoramidate, a stable, neutral analog, at the site of an amber stop codon in *E. coli*. They convert this unnatural amino acid to tyrosine phosphate under acidic conditions. Luo *et al.* adopt a similar strategy. They incorporate phopshotyrosine or its nonhydrolyzable phosphotyrosine analog 4-phosphomethyl-L-phenylalanine and increase the intracellular availability of these species by supplying them to cells in the form of a dipeptide that can be taken up by a transporter and subsequently hydrolyzed by an intracellular peptidase.

METABOLOMICS

Myers O D, Sumner S J, Li S, Barnes S, Du X. Detailed investigation and comparison of the XCMS and MZmine 2 chromatogram construction and chromatographic peak detection methods for preprocessing mass spectrometry metabolomics data. *Analytical Chemistry* 89;2017:8689–8695.

Myers O D, Sumner S J, Li S, Barnes S, Du X. One step forward for reducing false positive and false negative compound identifications from mass spectrometry metabolomics data: new algorithms for constructing extracted ion chromatograms and detecting chromatographic peaks. *Analytical Chemistry* 89;2017:8696–8703.

In this pair of papers, Myers *et al.* address the fidelity of peak detection in extracted ion chromatograms from untargeted metabolomics data acquired by liquid chromatography (LC)- mass spectrometry (MS) or gas chromatography (GC)-MS. The *centWave* algorithm, which uses the continuous wavelet transform method, has been incorporated into two widely used open-source software packages for processing untargeted metabolomics data, namely XCMS and MZmine 2. These two software packages yield substantial numbers of false-positive peaks and significant differences in peak lists from the same dataset. In the first of the two papers, the authors document features of data that produce false-positive and false-negative peaks in the two implementations of *centWave.* This information will help users use the packages more efficiently. In the second paper, they describe new algorithms for construction of extracted ion chromatograms and for peak detection. These algorithms yield fewer false-positive peak identifications, while performing as well as XCMS and MZmine 2 in detecting known peaks. The authors also contribute to discussion of the best way to specify mass tolerance in these analyses. Implementations of the new algorithms are made available online.

MASS SPECTROMETRY

Riley N M, Westphall M S, Hebert A S, Coon J J. Implementation of activated ion electron transfer dissociation on a quadrupole-Orbitrap-linear ion trap hybrid mass spectrometer. *Analytical Chemistry* 89;2017:6358–6366.

Riley N M, Hebert A S, Dürnberger G, Stanek F, Mechtler K, Westphall M S, Coon J J. Phosphoproteomics with activated ion electron transfer dissociation. *Analytical Chemistry* 89;2017:6367–6376.

Riley N M, Westphall M S, Coon J J. Activated ion-electron transfer dissociation enables comprehensive top-down protein fragmentation. *Journal of Proteome Research* 16;2017:2653–2659.

Electron transfer dissociation (ETD) is a widely used fragmentation method in proteomics. However, in ETD, precursor ions with low-charge density often undergo cleavage without product ion dissociation (nondissociative electron transfer). This curtails the yield of sequence-informative product ions. The effect may be overcome by the supply of more energy during the ETD reaction, most commonly by gentle collisional dissociation, but this increases duty cycle time and induces hydrogen abstraction. Supplemental infrared photoactivation can also accomplish the goal of improving product ion release, and this method has the added advantage of minimizing the time in which product ions are held together, thus diminishing hydrogen rearrangements. Even better, it does this without adding to cycle time. The present papers describe and characterize a new implementation of activated ion ETD using an infrared laser affixed to an Orbitrap Fusion Lumos mass spectrometer from Thermo Fisher Scientific (Waltham, MA, USA). This instrument has a quadrupole-Orbitrap-quadrupole linear ion trap (QLT) hybrid configuration. The hardware modification is accomplished with minimal reconfiguration. In the first paper, the authors show a near doubling of tryptic peptide identifications in LC-MS/MS experiments compared with ETD alone and demonstrate performance superior to other available supplemental activation methods. By combining activated ion ETD in the high-pressure cell of the QLT with short infrared multiphoton dissociation (IRMPD) activation in the low-pressure cell, they produce product ion spectra even richer in b/y-type and c/$z^{\bullet}$-type product ions. The second paper is devoted to analysis of phosphorylation sites. The improved fragmentation, especially of precursor ions with low-charge density, leads to identification of triple the number of phosphorylation sites in LC-MS/MS experiments than is achieved with ETD alone. Furthermore, the method produces phosphate neutral loss from all four types of ions, a feature that is exploited to improve localization of phosphorylation sites on phosphopeptides. The authors further demonstrate that the activated ion ETD method improves top-down phosphorylation site analysis of the multiply phosphorylated protein α-casein. In the third paper, they show near-complete sequence coverage in top-down analysis of proteins up to 16 kDa and substantial improvement in coverage compared with ETD alone or higher energy collisional activation following ETD for all proteins investigated. Additionally, the authors anticipate that the methodology will enable whole proteome analysis in the negative-ion mode.

Kottke P A, Lee J Y, Jonke A P, Seneviratne C A, Hecht E S, Muddiman D C, Torres M P, Fedorov A G. DRILL: an electrospray ionization-mass spectrometry interface for improved sensitivity via inertial droplet sorting and electrohydrodynamic focusing in a swirling flow. *Analytical Chemistry* 89; 2017:8981–8987.

The sensitivity of electrospray ionization is limited in part by radial expansion of the Taylor cone which results in under-sampling of analyte by the mass spectrometer inlet receiving the ions. Sensitivity is also limited by the passage through the inlet of droplets that are too large to emit desolvated ions but nevertheless contribute to background signal by supplying neutral particles. Kottke *et al.* here describe an interface that ameliorates these problems. It induces a swirling flow of droplets as they emerge from the electrospray emitter. The swirling motion mediates inertial separation of the droplets based upon their size. Larger droplets that are incapable of producing desolvated ions are removed, while smaller droplets are directed toward the mass spectrometer inlet. The consequent reduction in background produced an improvement of 10-fold in signal-to-noise ratio for angiotensin I during direct infusion nano-electrospray, and an improvement of 10-fold in the detection limit for angiotensin II. Large improvements in signal strength are also recorded in LC-MS mode.

PROTEINS—PURIFICATION AND CHARACTERIZATION

Di Trani J M, Moitessier N, Mittermaier A K. Measuring rapid time-scale reaction kinetics using isothermal titration calorimetry. *Analytical Chemistry* 89;2017:7022–7030.

Isothermal titration calorimetry (ITC) measures the increase in power (for endothermic reactions) or decrease in power (for exothermic reactions) that must be applied to a reaction cell containing a substance of interest when a molecule to which it binds is added in incremental amounts. The technique produces values for the affinity, enthalpy, entropy, and stoichiometry of the binding interaction. ITC can also be used to measure reaction kinetics. This requires analysis of the shapes of the response peaks in the time dimension—rapid reactions yield sharp peaks and slow reactions yield broad ones. With rapid reactions (seconds), the observed response is broadened by response parameters related to the instrumentation hardware and fluid mixing, and this broadening must be taken into account. Di Trani *et al.* here test existing instrument-response correction models and find them wanting. They substitute an empirical response function derived by performing very short burst injections to study a rapidly reacting molecular system

(Ca^{2+} binding to EDTA). With this new correction, they replicate known Michaelis-Menten parameters for the enzyme trypsin. They then analyze rapid reactions of prolyl oligopeptidase and interestingly, observe non-Michaelis-Menten behavior that has been overlooked in previous ITC and spectroscopic studies, thereby demonstrating the advantages of the methodology. The authors caution that correction functions are likely to vary with parameters, such as temperature, stirring speed, solvent viscosity, solution density, and thermal conductivity. Therefore, response correction functions should be derived under conditions as closely similar as possible to those of the binding interaction of interest.

Shuford C M, Walters J J, Holland P M, Sreenivasan U, Askari N, Ray K, Grant R P. Absolute protein quantification by mass spectrometry: not as simple as advertised. *Analytical Chemistry* 89;2017: 7406–7415.

Shuford *et al.* consider the use of various kinds of internal and external calibrants for quantification of proteins by mass spectrometric analysis of peptides derived by tryptic digestion of analyte proteins. The authors illustrate the inaccuracies that may be introduced in such measurements by differences in the extent of tryptic digestion of the calibrant and analyte proteins, and by the presence of different secondary modifications (and therefore, mass spectral signal splitting). The authors conclude that recombinant stable isotope-labeled standard proteins make better internal standards than stable isotope-labeled peptides, because they permit some sources of variation in digestion efficiency, as well as matrix effects, to be accounted for. But the authors also caution that inaccuracies may still arise from secondary modification of the calibrant protein, the analyte protein, or both. Therefore, investigators are counseled to treat all reference standards as surrogates/analogs of the analyte protein rather than chemically identical versions of it.

FUNCTIONAL GENOMICS AND PROTEOMICS

Price N D, Magis A T, Earls J C, Glusman G, Levy R, Lausted C, McDonald D T, Kusebauch U, Moss C L, Zhou Y, Qin S, Moritz R L, Brogaard K, Omenn G S, Lovejoy J C, Hood L. A wellness study of 108 individuals using personal, dense, dynamic data clouds. *Nature Biotechnology* 35;2017:747–756.

Leroy Hood, Nathan Price, and colleagues have launched an initiative, called the Pioneer 100 Wellness Project (P100), the goal of which is to collect longitudinal data for 100 "well" individuals to assess holistically genetic and environmental determinants of health and interactions between the two. The data are as comprehensive in scope as possible: they include whole genome sequences; clinical tests; and metabolomic, proteomic, and microbiome data sets. These data have been collected for each of 108 individuals at 3 time-points during a period of 9 mo, along with records of the participants' daily activity (the last with limited compliance). The present project is a pilot for an even larger study: the 100K Wellness Project (https://www.systemsbiology.org/research/100k-wellness-project/), a Framingham Heart Study-like analysis (www.framinghamheartstudy.org) of 100,000 individuals. The pilot study establishes data procurement and analysis methods for that larger project. The authors compute statistically significant inter-omic correlations and sort them by unsupervised clustering to reveal densely interconnected subsets or "data communities," both within and between time points. The data communities often represented clusters of physiologically interconnected analytes. The approach has led to the identification of candidate biomarkers, such as γ-glutamyltyrosine as a putatively improved biomarker for risk of diabetes independent of body mass index. The authors also identify molecular correlates of polygenic disease risk scores, calculated from published genome-wide association study data, to reveal possible ways in which genetic predisposition might be manifest in analyte changes. For example, genetic inflammatory bowel disease risk is found to be negatively correlated with plasma cytosine level. The project also has an interventional arm: behavioral coaching is provided to help participants improve clinical biomarkers. This practice provides opportunities to observe the effects of perturbations on health. The project has been criticized on a number of grounds, including the bias of its participant pool toward affluent whites and the lack of evidence for intervention based on the markers being measured. However, it is hoped that the work will continue to reveal connections for further investigation.

Ma H, Marti-Gutierrez N, Park S-W, Wu J, Lee Y, Suzuki K, Koski A, Ji D, Hayama T, Ahmed R, Darby H, Van Dyken C, Li Y, Kang E, Park A R, Kim D, Kim S-T, Gong J, Gu Y, Xu X, Battaglia D, Krieg S A, Lee D M, Wu D H, Wolf D P, Heitner S B, Belmonte J C, Amato P, Kim J-S, Kaul S, Mitalipov S. Correction of a pathogenic gene mutation in human embryos. *Nature* 548;2017:413–419.

In the context of human embryonic genome editing, the conversion of a disease-causing DNA sequence to a normal sequence in a specific, accurate, and efficient manner represents an exceedingly demanding application of the

CRISPR-Cas9 methodology. Ma *et al.* have investigated its feasibility in correcting defects in the *MYBPC3* gene in human embryos. This research is ethically and legally acceptable under existing guidelines and regulations, although NIH funds cannot be used to support it. The embryos were never intended for implantation. The *MYBPC3* gene is expressed in cardiac myocytes and encodes myosin-binding protein C, which functions in the maintenance of sarcomere structure and in the regulation of contraction and relaxation. Defects in the gene are associated with autosomal-dominant inheritance of hypertrophic cardiomyopathy. The experiments of Ma *et al.* aim to correct the sequence of a mutant allele derived from the father's sperm in a fertilized egg, where a normal allele derived from the mother's oocyte is also present. They microinject a guide RNA bound to the Cas9 protein to stimulate the formation of a double-stranded break at the site of the sequence defect. They accomplish its homology-directed repair using the homologous, normal chromosome as template. They observe that when they supply exogenous DNA molecules (marked by codon-usage variants) as repair templates, these templates are actually used much less frequently than the endogenous chromosome when the sequence is corrected. The authors focus on three cardinal issues. The first is the efficiency of repair procedure, which must be high to avoid the formation of mosaic embryos in which some cells are corrected, and others not. With the injection of genome-editing components into the oocyte at the same time as sperm, they are able to correct the sequence in 42 of 58 embryos (72.4%). Only one of the 42 embryos is found to be mosaic. The second issue is the specificity of the procedure, which must be high to avoid off-target sequence alterations. The authors detect none. The third issue is the accuracy of the procedure to avoid indels resulting from the competing but less accurate repair process of nonhomologous end-joining. The authors find evidence for operation of this undesirable process in 16 of the 58 embryos that they analyze. The high levels of efficiency and accuracy achieved in this study are further milestones in the adoption of genome editing to clinical practice.

Rheinbay E, Parasuraman P, Grimsby J, Tiao G, Engreitz J M, Kim J, Lawrence M S, Taylor-Weiner A, Rodriguez-Cuevas S, Rosenberg M, Hess J, Stewart C, Maruvka Y E, Stojanov P, Cortes M L, Seepo S, Cibulskis C, Tracy A, Pugh T J, Lee J, Zheng Z, Ellisen L W, Iafrate A J, Boehm J S, Gabriel S B, Meyerson M, Golub T R, Baselga J, Hidalgo-Miranda A, Shioda T, Bernards A, Lander E S, Getz G. Recurrent and functional regulatory mutations in breast cancer. *Nature* 547;2017:55–60.

Cancer genomes may contain very large numbers of mutations, but the number actually contributing to the malignant phenotype—the driver mutations—is often quite small. Most of the driver genes discovered to date have been in coding regions, suggesting that noncoding regions are less important. Rheinbay *et al.* present data that challenge this suggestion. They argue that by analyzing relatively small numbers of tumors of any particular type, searches have hitherto lacked the statistical power to identify driver mutations in noncoding regions, as the sequence search space in noncoding DNA is exceedingly large and therefore, incurs an inordinate multiple-testing penalty. Therefore, the authors restrict their search to promoters, defined for the purposes of this study as sequences between 400 bp upstream and 250 bp downstream of annotated transcription start sites. They sequence (to a depth of 80 fold) 360 primary breast tumors and patient-matched normal samples. The authors identify significantly recurrent mutations in the promoters of 9 genes. One of these, *FOXA1*, is a known driver of hormone receptor-positive breast cancer, and another two, *RMRP* and *NEAT1*, encode nontranslated RNAs that are shown to affect protein-expression levels. Such findings are potentially useful for personalized clinical intervention. The number of patients carrying mutations in promoters was lower than for coding regions, but the mutation rates were similar when corrected for target size. The lower frequency of driver mutations thus far discovered in noncoding regions may therefore be a result, at least in part, of their smaller functional genomic footprint.

IMAGING

Muller L, Baldwin K, Barbacci D C, Jackson S N, Roux A, Balaban C D, Brinson B E, McCully M I, Lewis E K, Schultz J A, Woods A S. Laser desorption/ionization mass spectrometric imaging of endogenous lipids from rat brain tissue implanted with silver nanoparticles. *Journal of The American Society for Mass Spectrometry* 28;2017:1716–1728.

Refinements of the Woods group's lipid-imaging methodology are reported in this paper. The technique is based on the use of silver nanoparticles as a matrix for laser-desorption/ionization MS. Size-selected silver nanoparticles are implanted into unfixed tissue sections with a nanoparticle implanter from Ionwerks (Houston, TX, USA). As the implantation process is uniform and dry, it avoids problems of irreproducibility and inhomogeneity and loss of spatial resolution encountered in spraying solutions of organic matrices, as performed in conventional matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). Desorption of lipids is accomplished with

a 337 nm laser. High-resolution images are recorded in positive ion mode for the distribution of galactoceramides, diacylglycerols, ceramides, phosphatidylcholines, and cholesteryl esters. Images for phosphatidylethanolamides, sulfatides, phosphatidylinositol, and sphingomyelins are recorded in negative ion mode. The authors caution that whereas antigen-cationized ceramide and diacylglycerol ions are detected as intact molecular ions, glycerophospholipids preferentially lose their unique head groups to form diacylglycerol, and sphingolipids lose their head group to form ceramide, so signals from these species may be derived from multiple precursors.

CELL BIOLOGY AND TISSUE ENGINEERING

Shaffer S M, Dunagin M C, Torborg S R, Torre E A, Emert B, Krepler C, Beqiri M, Sproesser K, Brafford P A, Xiao M, Eggan E, Anastopoulos I N, Vargas-Garcia C A, Singh A, Nathanson K L, Herlyn M, Raj A. Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. ***Nature*** **546;2017:431–435.**

The elucidation of the ways cancer cells acquire resistance to therapeutic agents that target signaling pathways informs the selection of new avenues for therapeutic targeting. Shaffer *et al.* study a paradigmatic example of resistance to chemotherapy: melanoma cells bearing the oncogenic V600E mutation. These cells initially respond to vemurafenib, an inhibitor of the BRAF protein, but later, a small subset of cells resistant to the drug leads to relapse. Shaffer *et al.* show that rare drug-resistant melanoma cells are present in culture even before drug exposure. They arise, not as a result of heritable mutations conferring resistance but as a result of sporadic variability in transcriptional activity of resistance genes at the single-cell level. Some cells show high expression levels of several resistance genes at once in patterns that indicate coordinate regulation of resistance genes. These cells are more likely to become resistant upon exposure to the drug. However, the phenotype is initially not heritable but rather reversible. The latently resistant cells acquire, by a transcriptional reprogramming mechanism, a stably resistant phenotype upon drug exposure. This reprogramming is associated with a reduction in occupancy of sites that bind the transcription factor SOX10 and an increase in occupancy of sites that bind the transcription factors JUN and/or AP-1 and TEAD. The reversibly resistant state is similar to that described previously by Sharma *et al.* (*Cell* 141;2010:69–80). Cancer types, other than melanoma, also exhibit sporadic coexpression of many of the same resistance genes, suggesting that the mechanism may be of general significance. Further investigation of this plasticity and reprogramming may open new possibilities for therapeutic targeting.

DRUG DISCOVERY AND SYNTHESIS

Stewart E, Federico S M, Chen X, Shelat A A, Bradley C, Gordon B, Karlstrom A, Twarog N R, Clay M R, Bahrami A, Freeman Iii B B, Xu B, Zhou X, Wu J, Honnell V, Ocarz M, Blankenship K, Dapper J, Mardis E R, Wilson R K, Downing J, Zhang J, Easton J, Pappo A, Dyer M A. Orthotopic patient-derived xenografts of pediatric solid tumors. ***Nature*** **549;2017:96–100.**

Models of human tumors established for the testing of therapeutic interventions have commonly taken the form of cell lines derived from human tumor cells and grown *in vitro* or tumors of murine origin analogous to the human malignancies that are maintained in mice. The growing of human tumor cells as xenografts in immunodeficient mice is an alternative approach that affords significant advantages over these models in the ease with which tumors can be generated and maintained. The cells can be engineered to express markers for tracking *in vivo*, and host animals can be altered to emulate the human tumor microenvironment in specific ways. The cells can be grown orthotopically, *i.e.*, within a murine tissue comparable with the human tissue of their origin, and they can be frozen and thawed for transplantation into new animal hosts at will. In the present paper, Stewart *et al.* document extensive use of orthotopic xenograft models of solid tumors to capture the complexity and diversity of 12 types of cancer among 168 individual pediatric patients. The authors perform genomic, epigenomic, and gene-expression profiling of the model tumors and perform immunohistochemical studies to determine the fidelity of their models to the patients' tumors. They perform drug screening to identify potential susceptibilities to therapy and identify drug vulnerabilities. The authors hope their work will encourage investigators to use orthotopic xenografts better to understand clonal selection in cancer cells, particularly for recurrent pediatric tumors.

Cole K P, Groh J M, Johnson M D, Burcham C L, Campbell B M, Diseroad W D, Heller M R, Howell J R, Kallman N J, Koenig T M, May S A, Miller R D, Mitchell D, Myers D P, Myers S S, Phillips J L, Polster C S, White T D, Cashman J, Hurley D, Moylan R, Sheehan P, Spencer R D, Desmond K, Desmond P, Gowran O. Kilogram-scale prexasertib

monolactate monohydrate synthesis under continuous-flow CGMP conditions. *Science* 356;2017:1144–1150.

In the pharmaceutical industry, small-molecule drugs are traditionally manufactured in large batches using big reactors. This mode of operation is ill suited to the production of high-potency compounds that are used in small amounts and require strict containment to protect plant personnel. It is likewise problematic for production of small batches to be used in treating limited numbers of patients in the context of clinical trials. By contrast, small-volume, continuous manufacturing of drugs on the smaller scale of 3–15 kg/d represents a more flexible approach that is better suited for producing pharmaceuticals on demand. This methodology offers numerous advantages of scale, and the U.S. Food and Drug Administration is supportive of it, in principal. Proof-of-concept reports of continuous manufacturing processes have appeared, but their deployment with rigorous control strategies is less common. The present paper, by contrast, describes continuous manufacture of prexasertib monomesylate monohydrate in compliance with current good-manufacturing practices. Prexasertib is an inhibitor of checkpoint kinase 1, which regulates DNA replication and the repair of damaged DNA. The drug induces DNA damage ($IC_{50} < 1$ nM *in vitro*), and replication catastrophe ensues. The drug is presently being assessed in Phases 1b and 2 clinical trials for use as a monotherapy or in conjunction with cytotoxic chemotherapy. It has previously been manufactured in a 9-step process but is here synthesized in a 7-step procedure on a scale of 3 kg/d using small, continuous reactors, extractors, evaporators, crystallizers, and filters, all situated in laboratory fume hoods. Advantages in efficiency, waste production, and safety are realized. It is anticipated that manufacturing practices of this kind will become commonplace in the near future.